Somites in Developing Embryos

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MODELS OF SEGMENTATION

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INTRODUCTION

The formation of segmented structures is a very important step during development of higher organisms. With the formation of somites in vertebrates or the segments in insects the primary anteroposterior pattern of the organisms is laid down. Segmentation is the result of the superposition of two pattern formation processes. One generates a periodic pattern, i.e. a repetition of homologous structures. It consists in vertebrates of somites and somitic clefts and in insects of segments and segment borders. Superimposed on this periodic pattern is a sequential pattern which makes the repetitive subunits different from each other. In recent years, we have proposed molecularly feasible models which are able to generate periodic and sequential structures precisely superimposed on each other (Meinhardt, 1982a,b). For insect development more detailed experimental and genetic data are available. For that reason the model of insect segmentation is more advanced. At the beginning of this paper a short overview of the model proposed for insect segmentation will be provided. I will show that, with some adaptations, the basic mechanism is also able to account for somite formation.

THE PROBLEM OF POLARITY IN PERIODIC STRUCTURES

The simplest periodic pattern would consist of the alternation of two cell states, for instance ...APAPA... The two compartments found in the thoracic segments of Drosophila or the different behaviour of the anterior and posterior parts of somites (Keynes and Stern, 1984) suggest such a model. However, in a binary sequence the position of a segment border or of a somitic cleft is not determined. It could be ..AP/PA/.. or ..A/PA/PA/... These patterns would have opposite polarity. One possible solution of this grouping and polarity problem would be that a third cell state exists, i.e. that each unit of repetition consists of three elements, for instance ..P/SAP/SAP/S.. In contrast to a repetition of two cell states, the serial repetition of three cell states leads to a defined polarity. The confrontation between two of the three cell states could be used as the signal to form a segment border or a somitic cleft. The other possibility would be that a suprastructure exists, for instance, ...OEOE... (odd and even) and each of these elements becomes further subdivided into A and P. In this case, the signal for a border or a cleft would be an OE confrontation but not an AP confrontation. For insects
experimental evidence strongly supports the first possibility (Meinhardt, 1984a). Grafting experiments with epidermis from an anterior (S) to a posterior (P) position or vice versa lead with high frequency to the formation of a (P/S) segment border (Wright and Lawrence, 1981), even if this transplantation is made within the same segment, in agreement with the first possibility. For somites, to my knowledge, no experiments are available to decide between these two possibilities. The experiments of Keynes and Stern (1984) suggest that already in the presomatic mesoderm a periodic pattern of at least two cell types is determined but this, as outlined above, does not answer the question of what determines polarity of the somites and the position of the cleft.

INSECT SEGMENTATION

The recently found mutations which lead to a change in the pattern of segmentation in Drosophila (Nüsslein-Volhard and Wieschaus, 1980) provide insights into the sequence of events which leads to the precise arrangement of segments. According to a model which accounts for the major phenotypes, segmentation is achieved in four major steps (Meinhardt, 1985, 1986). (i) Under the control of maternal genes, a gradient is formed within the egg which has its high point at the posterior egg pole. (ii) Under its influence, a coarse subdivision into four cardinal plus two marginal regions takes place. (iii) The border between any two cardinal regions organizes the first truly periodic pattern, the double segment pattern. It consists of a serial repetition of four cell states, termed 1, 2, 3 and 4. (iv) The segmentation proper results, as mentioned, from the repetition of three cell states, the classical compartments A (anterior) and P (posterior) and a third region S at the anterior side of each segment. A segment border is formed whenever P and S cells are juxtaposed (...)S/SAP/SAP/S...). In other words, it is not that an originally homogenous segment becomes subdivided into three parts but that the segments proper and their separation from neighbouring segments result from this threefold repetition. The double segment (...)1234...) to single segment transition results from an inductive process in which one 1234 sequence give rise to two SAP sequences. According to this view, insect segmentation results from an interlinked series of pattern forming processes. It starts with a simple gradient, passes through the coarse pattern of cardinal regions, produces the double segment pattern and ends up with the single segment pattern. Each pattern has a higher complexity or a higher spatial resolution than that by which it has been induced.

The use of a border to induce a finer structure does not end with the formation of a segment border at the P/S confrontation. The A-P border is used in a similar way to determine position and polarity of a new structure. An A-P border is a prerequisite to form a leg or a wing (Meinhardt, 1983a). For that reason, a leg can never appear on a segment border.

The use of borders between existing structures to determine the next finer pattern enables the determination of the new structure in the correct position, handedness and orientation in relation to the existing structures. The use of borders and their intersections seems to be a general mechanism in the determination of substructures. Many experiments dealing with formation and regeneration of vertebrate limbs can be explained using this assumption (Meinhardt, 1983b).

The segments in Drosophila appear more or less simultaneously. In other insects, for instance in crickets (see Sander, 1981) the segments appear in an anteroposterior order. The sequential pattern formation seems to be the more elementary form since the segments are formed in this mode in the more primitive Annelids. As will be explained below in detail, for the sequential segmentation fewer molecular interactions are required.
The somites form sequentially in an anterior to posterior order. Many experiments have provided insights into how somite formation is controlled. The wave of somite formation does not result from a series of inductive events from the last somite formed onto the next somite to be formed (Deuchar and Burges, 1967; Pearson and Elsdale, 1979). This can be concluded from experiments with isolated pieces of presomitic mesoderm separated from the embryo before somite determination. The somites form at the correct time, obviously without a trigger from the anterior neighbour. A graded tissue property seems to exist which causes somite formation in a count-down like process. This count-down is finished much earlier in the anterior part. The wave-like appearance results therefore from an ordered sequence of count-down terminations. On the other hand, perturbations of somite formation induced by a short heat shock lead to pattern abnormalities which are much broader than that expected from the duration of the heat shock itself. This indicates that an irregularity formed in one somite induces another irregularity in the next following somite. At least over the distance of some somites, neighbourhood-relations play a role.

The somites are not a homogeneous block of tissue. As mentioned, Keynes and Stern (1984) have shown that each somite consists at least of an anterior and a posterior part. The polarity of the future somites is already fixed before the separation of the somites since a rotation of a corresponding graft leads to somites with reversed polarity; i.e., the polarity of the somites is maintained with respect to the original anteroposterior axis. This indicates that it is not that first a homogeneous somite is formed which becomes further subdivided in a second step.

Despite the fact that the anterior somites appear much earlier than more posterior somites, the sizes of the somites are regulated in relation to the total size. Cooke (1981) has shown that in amphibians only the first ca. 20 somites are size-regulated, while the more posterior somites are smaller and size-independent.

The somites are presumably different from each other since the constituents of the somites (sclerotomes, dermatomes, myotomes) give rise to different structures of the body along the anteroposterior axis.

In summary, a model which would account for somitogenesis has to account for the following features: (i) A periodic structure is formed in an anterior to posterior order. (ii) The time at which the separation of somites occurs is cell-externally determined. (iii) Interactions of neighbouring cells are involved in the generation of the periodic structure. (iv) Each somite consists at least of an anterior and a posterior part. (v) The size of the anteriormost (about 20) somites is regulated to the total size of the organism, the remaining somites are smaller and independent of the size of the organism. (vi) The somites formed in this process are different from each other.

The model I have proposed has these features due to the interaction of the following components: A gradient is generated in the organism which has the high point at the posterior side of the embryo. Under the influence of this gradient, cells start to oscillate between (at least) two cell states, A and P. The number of oscillations is controlled by the local morphogen concentration. The cells count the number of oscillations between A and P. With each oscillation the somite which is eventually formed achieves a more posterior specificity.
HOW TO GENERATE POSITIONAL INFORMATION

The initiation of somite formation at the anterior end of the organism and the region-specific time of somite formation require some sort of positional information. I assume a graded distribution of a morphogen which has its high point at the posterior end of the embryo, in analogy to the situation in insects (see Meinhardt, 1977). We have shown (Gierer and Meinhardt, 1972, Meinhardt, 1982a) that a graded concentration profile can be generated by local autocatalysis and long-range inhibition.

Somite formation requires not only positional information along the anteroposterior but also along the dorsoventral axis to enable the separation of presomitic mesoderm from the pronephric zone and from the lateral plate. It is essential for a developing organism that these two patterns are not parallel. The best separation of both axes would be achieved if both patterns were oriented perpendicular to each other. This requires some coupling between both pattern forming systems. Despite the importance of this problem, very little is known about how axes are kept orthogonal to each other. A possible model would be an early subdivision of an embryo into a dorsal and a ventral half and that the anterior-most and posterior-most region of the embryo have to lie on the border between these two halves (see Meinhardt, 1984b).

HOW TO GENERATE A PERIODIC PATTERN

As mentioned, the results of Keynes and Stern (1984) indicate the early formation of a periodic pattern consisting of the alternation of at least two cell states. For the sake of simplicity the model for somite formation will be explained under the assumption that the periodic pattern is composed of only two cell states, to be called A and P. As mentioned, this is certainly an oversimplification and either three cell states or a suprastructure (or both) must be involved.

A periodic stripe-like arrangement of regions in which either a substance A or a substance P is present in high concentration can appear if the following conditions are satisfied: (i) The substance A and the substance P are, directly or indirectly, autocatalytic. (ii) The production of A and P is locally exclusive; i.e., if in a particular cell A is produced at a high rate, the production of P is suppressed and vice versa. (iii) On long range, the production of one substance depends on the production of the alternative substance in the neighborhood. In other words, on long range the A and P production mutually reinforce each other, for instance via diffusible co-factors. The most obvious level of such an interaction involving auto-regulation, mutual repression and crosscatalysis is the gene level. The A- or P-production would be directly under the control of corresponding genes. If a particular gene is active in a particular cell we will say this cell is in a particular cell state.

Such an interaction consisting of local autocatalysis, short range exclusion and long range activation has pattern forming capabilities. If no other developmental constraints are imposed the pattern would emerge simultaneously in an extended field of cells (Fig.1). However, the resulting pattern will be somewhat irregular since the pattern which emerges in one region is to a large extent independent of that emerging at a distant position because the coupling via the diffusible co-factors would be too slow. From the model, this irregularity is expected whenever a periodic pattern appears in a field which has an extension that is large when compared to the distance between single elements. In other developmental situations, such irregularities of periodic patterns are well-known. The stomata of leaves or the arrangement of cilia on early Xenopus embryos are examples.
Fig. 1 Formation of a periodic pattern. (a) Scheme of a reaction which is able to generate periodic patterns of two cell states. A and P are autocatalytic substances; the autocatalysis is presumably realized on the gene level. Both feedback loops compete with each other, for instance, via a common repressor. This competition is cell-local. On long range, both feedback loops activate each other via $S_A$ and $S_P$. (b) Stages in the formation of a periodic pattern. The spatially homogeneous distribution of A and P is unstable. Small random fluctuations are sufficient to initiate pattern formation. In a particular cell, either the A loop or the P loop will become active and the other will be suppressed. Due to the mutual activation, an A cell cannot appear too distant from a P cell and vice versa. The resulting pattern is a somewhat irregular alternation between A and P regions.

Fig. 2 Generation of a regular ..APAP.. pattern by oscillations and border formation. (a) Initial condition: a primary AP border is present. Cells around this border stabilize each other in the A or the P state. (b-d) Cells at a distance switch to the other state, creating in this way a new border. Each full cycle of the oscillation creates one AP pair. The APA.. oscillation comes to a rest if the total field is subdivided into A and P regions. This pattern is more regular since a new border appears always at a certain distance from an existing border. It is stable in time since cells of one type are close to cells of the other type – an arrangement that enables an efficient mutual stabilization.
More regular patterns emerge if the pattern forming mechanism is at work during growth since new elements are added whenever the distances to the existing one become large enough. An example is the regular arrangement of leaves on an outgrowing shoot.

For the model envisaged to generate a regular periodic structure in a large field (such as required for somite formation) another property of the long range activation - short range exclusion mechanism is of importance: the tendency to oscillate. Let us regard the behaviour of an isolated cell in state A (i.e. a cell in which genes responsible for the synthesis of a substance A are active). Then, the (suppressed) P state becomes activated by the helping co-factors produced by the (active) A state. In turn, the A-state is not supported since no active P-state is present in the vicinity. Thus, the cell will switch from the A-state into the P-state and, after some time, for the same reason back to A, and so on. The same type of oscillation can occur in a larger group of cells as well if all cells are at a particular time in the same cell state. However, if for any reason an initial A-P border has been formed, the formation of further borders and ultimately a spatially stable pattern "crystallizes" around this border since, around this border, the A and P cells stabilize each other. At a distance however, at which this stabilizing influence is insufficient, cells continue to oscillate. Each switch to the other state creates a new border around which the cells become stabilized (Fig. 2). With each oscillation the fraction of the cells incorporated in a stable A-P pattern grows at the expense of the cells which still oscillate between A and P until all cells are integrated in this spatially periodic pattern which is stable in time.

How can the first boundary arise? The obvious means to assure an overall control would be a morphogenetic gradient. Let us assume that initially all cells are in the state P and that a certain threshold concentration is required to switch from P to A. All cells exposed to more than this threshold concentration will switch from P to A, creating in this way the first boundary. Cells distant from this boundary will switch back to P, creating the next (AP) border and so on. The formation of a stable PAPA ... pattern spreads out from the first border in a regular way (Fig. 3).

THE SUPERPOSITION OF SEQUENTIAL AND PERIODIC PATTERN

With the formation of somites, not only a periodic structure is formed. The somites are presumably different from each other since particular somites and their derivatives - dermatomes, myotomes and sclerotomes give rise to particular structures. Little is known for somites, how this superposition of the two patterns, the sequential (to be called 1,2,3, 4,5...) and the periodic, (..APAP.. or ..SAP/ SAP/..) is achieved. In insects it is clear that both patterns are precisely in register and that the formation of the periodic pattern is the primary event. The sequential pattern (1,2,3,4,5...) must be under control of the periodic pattern (see Meinhardt, 1982a, b). I will assume that the same is also true for somites. For the model I assume that in each of the first ca. 20 somites (AP pairs or /SAP/ units) a different gene (1,2,3..) is activated. The mechanism to be described below is able to generate a precise superposition of the two patterns.

It is a property of the model that for a particular cell there is a one-to-one correlation between the number of AP oscillations a cell has made in its history and the number of the A-P pairs to which it belongs, counting from the first P-A border. This correlation results from the fact that with each full AP cycle, one stable AP-pair is added (Fig. 2). Thus, a precise superposition of the two patterns results if the switching of P to A (or A to P) is used to activate a subsequent gene (Fig. 3). In this way, each AP pair would be different from its neighbour. An analogy provides a better intuition for the mechanism envisaged. The system may be compared
with a pendulum-escape mechanism of a grandfather clock. Lifting up the weights initiates the periodic movement of the pendulum. With each full cycle of the pendulum, the hand of the clock advances one unit. The periodic movement of the pendulum is the primary event, the sequential advancement of the hand is under its control.

Another analogy provides some intuition about how the sequential activation of control genes under the influence of the periodic change between two (or three) cell states may work. Imagine a ship in a channel system with locks. A lock can be in two states. Either the lower gate is open and the upper gate is closed or vice versa. In neither state can a ship

![Fig. 3 Stages in the generation of a periodic (..PAPAP..) and a sequential (12345...) pattern under control of a morphogen gradient. (a) the stable gradient. (b) Initial conditions: all cells are in the P state and gene 1 is turned on (high density of dots), X indicates a high concentration of a substance which is produced in the P state and that induces a transition to the next gene after a P-A switch. (c) All cells exposed to a certain morphogen concentration switch from P to A and, simultaneously, from gene 1 to gene 2. (d) The first PA border is formed. Around this border, the cells stabilize each other mutually in the A and the P state. At a distance, cells switch back to the P state. (e) A second (AP) border has been formed. At a distance (at a position at which sufficient morphogen is present) cells switch to A. This causes a gene 2 - gene 3 transition. (f) Final state. A regular periodic pattern and, precisely in register, a sequential pattern (1234...) have been formed. Each region in which a particular gene is active is subdivided into an anterior and a posterior (A and P) part. Each border between regions of different gene activities is also a (PA-) border in the periodic pattern.
pass through. But in one state the ship can enter into the lock and after the switch to the other state, the ship can pass. In one state, the transition is prepared but blocked. In the other state, the block is released, the transition can take place, but no preparation of the next transition is possible. For the sequential activation of control genes I assume that, for instance, in the P-state a substance X is produced that activates the subsequent gene, but that its action is blocked. In the A-state, the block is released but X is no longer produced. Only with a P-A transition the activation of the subsequent gene can take place due to the simultaneous

![Diagram](image)

**Fig. 4** Size regulation and pattern formation in posterior fragments. The upper subpictures show the assumed positional information, the central figures the oscillation between A(−−−) and P(XXX) as a function of time as well as the generation of a stable APAP... pattern. The lower subpictures show the final pattern of gene activities. The same mechanism as in Fig. 3 is assumed. (a,b) In fields of different sizes, similar patterns are generated if the concentration range of the gradient is regulated. The size of the individual AP pairs is a certain fraction of the total size. The broken line indicates the transition from an oscillatory behavior to stable pattern formation (i.e. the time of somite determination). (c) In a posterior fragment (low gradient levels are missing) the first oscillations occur in synchrony without border formation. During these oscillations, activation of more posterior genes takes place which increases the threshold for further P-A transitions. Eventually, the formation of the stable pattern occurs at the same time and at the same position as it would happen in the undisturbed system. This simulation demonstrates that, in agreement with the experiments, no trigger from the anterior side is required (after Meinhardt, 1982a).
release of the block and the presence of the substance X. In contrast, activation of subsequent control genes can not occur if cells remain permanently in the P- or in the A-state.

To make this model compatible with the experimental results I have to assume that not only the formation of the first P-A border is under gradient control but that with each switch to a subsequent gene the threshold for the next P-A transition is increased too. If the driving gradient is size-regulated, within certain limits the resulting periodic pattern adapts to the total size of the organism (Fig.4a,b). Thus, the local morphogen concentration determines how many oscillations a cell has to undertake until the stable periodic pattern appears and, simultaneously, which particular type the future somite will be. To use the picture of the grandfather clock once again: the level to which the weights have been lifted (local morphogen concentration) is decisive of how many oscillations the pendulum will make and therefore, at which position the hands will come to a rest. With this assumption the model has the count-down character mentioned in the introduction. According to the model, in an isolated posterior piece, initially the cells oscillate synchronously and obtain in this way a more posterior determination (Fig. 4c). In this process, the threshold for the P-A oscillation is increased successively until at the anterior side of the fragment the gradient is insufficient to drive the oscillation further and the first stable PA-border is formed. Implicit in this model is that the gradient is stable and does not change due to the isolation of the fragment.

Fig.5 Formation of a sequential/periodic pattern in a growing field of cells. This model describes the formation of posterior somites in a budding zone. Growth is assumed to take place at the right side of the field. New cells are in the same cell state as their neighbours. (a-d) Whenever the distance to a boundary becomes too large, a switch into the alternative cell state takes place. In (c), the transition of the terminal cells from P to A is depicted. With each P-A transition an activation of the subsequent gene takes place. The result is a periodic pattern (. . .APAP. . .) and, precisely in register, a sequential pattern (12345. . .) (after Meinhardt, 1982a, where also computer programs for these simulations can be found).
The activation of different control genes may be required only for the
determination of the more anterior somites because these have to form
different structures (for instance ribs). In contrast, the somites that form
later in the tail may be identical. Experimentally, it has been found that
only the first ca. 20 somites adapt to the changes in size (Cooke, 1981). In
terms of the model, this suggests that only with the formation of the first
ca. 20 somites an increase in the A-P-oscillation threshold take place. If
the threshold is not increased, the A-P-A pattern is formed at the closest
possible distance. This is in agreement with the observation that the
non-size regulating posterior somites are always smaller than the more
anterior ones.

Experimentally it has been found that the anterior somites result from
the separation of the existing cells in the presomitic mesoderm while the
more posterior somites result from a zone of cell proliferation in a budding-
like process. These two modes are explicable by the model in a straightforward way. Imagine an APA... pattern growing at one margin. If, for instance, cells in the A-state at the growing tip become too remote from P cells such that they are no longer sufficiently stabilized by the P cells, the A cells
at the tip switch to P. For the same reason, after sufficient growth of the
new P-region, the distant P cells will switch to A, and so on (Fig.5).

Cooke and Zeeman (1976) have proposed a model for somite formation
according to which an oscillator gates a wavefront. In the model I propose,
the oscillation (between A and P), the wavefront (separating the oscilla-
ting and the stable pattern) as well as the spatially stable periodic pattern (of A and P) result from one and the same mechanism. In addition,
the model I propose accounts for the fact that the somites become different
from each other.

**EXPERIMENTAL TEST AND OPEN PROBLEMS**

The model described above is an attempt to find a feasible molecular mecha-
nism compatible with available data. This model would obtain strong
support if the postulated oscillations in the mesoderm before somite forma-
tion could be detected. One full cycle of this oscillation should take
precisely the same time as that required for the formation of one somite
(about 2 h in Xenopus).

The model has been explained under the assumption that an alternation
between two cell states exists. As mentioned, the explanation of the forma-
tion of the somitic cleft requires the alternation between at least three
cell states or a suprastructure. In insects, good evidence exists for both,
the threefold subdivision of segments and the suprastructure with the length
of repetition of a double segment (Nüsslein-Volhard and Wieschaus, 1980). In
Drosophila, an even coarser pattern exists in the form of the cardinal
regions. It is not known whether a similar coarse pattern is first laid
down in vertebrates. If yes, I would expect that these are intimately
connected with the regions involved in limb formation. Extending classical
experiments, Slack (1976) has found a competent and a polarizing zone
required for limb formation. I have shown (Meinhardt, 1983b) that many
experiments can be accounted for under the assumption that positional
information for limb formation is generated at this border (or, more
precisely, at the intersection of this border with a dorsoventral border).
The extension of these zones is about 3-4 somites. If any cardinal regions
exist in vertebrates I would expect that these are identical to the zones
found for limb formation.
From the model I expect that the genes involved in the formation of periodic structures have an autocatalytic feedback on their own activation and show mutual competition. On long range, these genes either activate each other or have a self inhibitory component. Both possibilities are equivalent since in systems with competition, selfinhibition is equivalent to direct enhancing the competitor. It could be that the recently discovered homeobox is involved in these functions.

I hope that this model helps in unravelling the molecular mechanism on which segmentation is based.

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